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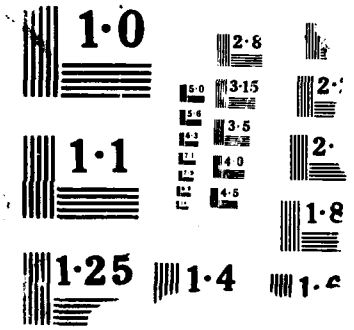
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This research pursues our previous observation that films of cytochrome c_3 deposited on a gold substrate undergo a change in resistivity of ten orders of magnitude when this tetraheme protein passes from a fully oxidized to a fully reduced state. This unusual behavior appears to be attributable to the ability of the four hemes to "communicate" through intramolecular as well as intermolecular electron transfer. To understand this phenomenon in more detail, four cytochromes c_3 with differing physical properties have been selected: 1. D.vulgaris (Miyazaki); 2. D. vulgaris (Hildenborough); 3. D. sulfuricans (Norway) and 4. D. gigas. The macroscopic redox potentials for each of the hemes in the four proteins have been determined. Electrostatic field maps based on the x-ray structures of the Norway and Miyazaki proteins have been prepared to assist in the design of homogeneous and heterogeneous electron transfer experiments. In the next year, the electrical properties of cytochrome films will be evaluated through Hall effect measurements in particular to establish whether the conductivity is ionic or electronic. Keywords: oxidation reduction reactions

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PROGRESS REPORT ON CONTRACT N0014-88-K-0055

Principal Investigator: George S. Wilson (Univ. of Kansas)
Michael A. Cusanovich (Univ. of Arizona)

Contracts: University of Kansas
Contract Title: Cytochrome Electron Transfer and Biomolecular Electronics
Start Date: 15 November 1987
Research Objective: To examine the homogeneous and heterogeneous electron transfer reactions in cytochromes containing multiple heme centers. Intramolecular and intermolecular e.t. rates will be measured as well as the electrical properties of protein films.

Introduction

This project was inspired in large measure by the observation of H. Sagara (M.S. Thesis, 1986, Yokohama National University) that films of cytochrome C_2, a tetraheme protein, showed a 10 order of magnitude change in resistivity when reduced from the fully oxidized to the fully reduced form. It had further been shown by Professor Katsumi Niki's group at Yokohama National University that this class of proteins exhibited very rapid electron transfer at electrodes, faster than any other class of proteins and can be can be examined by direct electrochemical techniques and the macroscopic potentials of the individual heme groups measured. It is assumed that the unusual ability of this protein to shuttle electrons through absorbed films can be attributed to the ability of the heme groups to communicate through intramolecular interactions. The arrangement of these groups is shown in Fig. 1 which is based on the x-ray structure of cytochrome C_3 (Miyazaki)

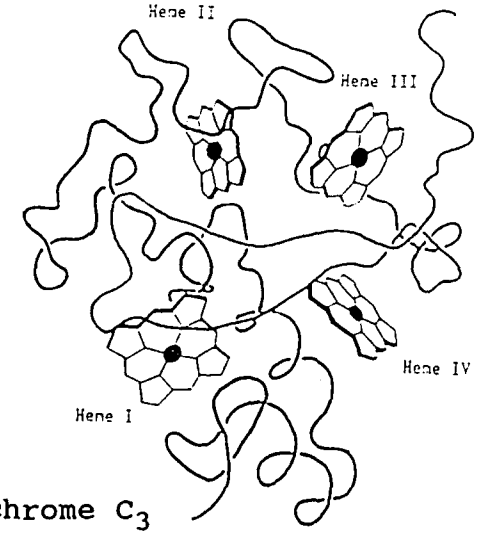

Figure 1 - Structure of Cytochrome C_3

Four cytochromes C_3 have been selected for study: 1. D. vulgaris (Miyazaki) DvM); 2. D. vulgaris (Hildenborough) (DvH); 3. D. sulfuricans (Norway) (DdN) and 4. D. gigas (Dg). They have been

so chosen either because their x-ray structures are known or because they have clearly different physical or chemical properties. As homogeneous electron transfer reaction rates are easier to define and measure, such studies will initially be used to characterize these systems. Further electrochemical studies will involve the use of differential pulse polarography (DPP) and other electrochemical pertubation techniques. In order to explain the high conductivity of cytochrome c films and their dependence on redox state, more detailed electrochemical and electrical measurements on the protein films will be needed. In particular it is necessary to establish whether the conductivity is primarily ionic or electronic. Structure-reactivity relationships will be examined by considering the differences among the various target molecules. In anticipation of the need for systematic variation of protein structure, we have established a collaboration with Dr. Gerritt Voordouw at the University of Calgary (Alberta) to find a vehicle for expressing the DvH gene which has already been isolated. An important link between the homogeneous solution properties and the properties of absorbed films will be established by spectroscopic measurements. In particular Surface Enhanced Raman Spectroscopy (SERS) and Surface Enhanced Resonance Raman Spectroscopy (SERRS) as well as infrared IRRAS and uv-vis reflectance techniques (ER) will be employed.

As the scientific organization of this project is somewhat complex, it will be reviewed briefly. The research is being conducted at three locations with the principal responsibilities indicated below.

*1. George S. Wilson - Department of Chemistry, University of Kansas - Electrochemical measurements of proteins and protein films especially DPP and impedance. Preparation and characterization of cytochrome C_3 - derived peptides. Chemical modification of cytochromes.

*2. Michael A. Cusanovich - Department of Biochemistry, University of Arizona - Preparation of purified cytochromes, homogeneous electron transfer studies using flash photolysis and stopped - flow techniques. Site-directed matagenesis.

**3. Katsumi Niki - Department of Physical Chemistry, Yokohama National University, Yokohama, Japan - Electrochemical measurements of proteins and protein films, Electrical properties such as the Hall effect. Spectroscopic measurements - IRRAS, SERS, SERRS, ER. Preparation of Langmuir - Blodgett films.

*Supported directly by ONR. **Collaborator.

PROGRESS (Year I) - Although the official start date was November 15, 1987, we were not able, owing to a variety of administrative delays, to begin the research until February 1988. Thus the Progress report describes the first 6 months of activity. Professor Cusanovich's

laboratory has prepared 10-100 mg of DvM and DvH and samples have been sent to Japan for the various studies to be carried out there. It is anticipated that adequate supplies of DdN and Dg will shortly be available. Preliminary experiments on carbon monoxide binding to DvH have been initiated. The results suggest that each of the four hemes has distinct kinetic properties and that transient intermediate states can be observed. These studies are important because they provide specific information about the chemical environments of each of the hemes.

The measurement of the macroscopic potentials of D. gigas was carried out in Prof. Niki's laboratory to complete the evaluation of the target molecules. The experimental and simulated results are shown in Figure 2.

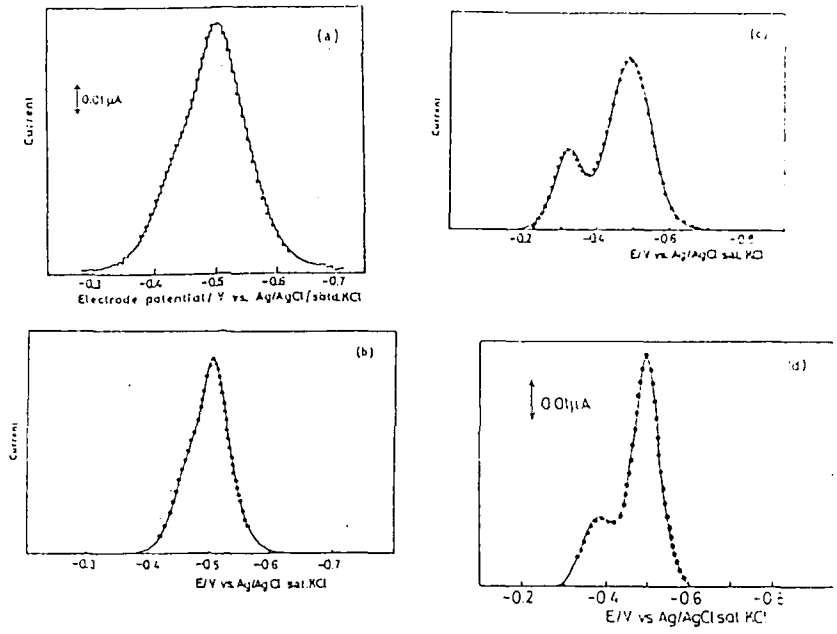

Figure 2 - Differential pulse polarograms of cytochrome c_3

a) DvM: Desulfovibrio vulgaris Miyazaki F (solid line, measured; dots, simulated)
b) DvH: Desulfovibrio vulgaris Hildenborough (dots, measured; solid line, simulated)
c) DdN: Desulfovibrio desulfuricans Norway (dots, measured; solid line, simulated)
d) Dg: Desulfovibrio gigas (dots, measured; solid line, simulated)

For reasons which are not yet clear, the existence of the distinct first peak indicates a substantial difference in potential between the first heme and the other three in the case of DdN and Dg. If the x-ray structures of DdN and DvM are superimposed, there are virtually no differences in the relative positions of the four heme

rings. There is substantial structural homology even though the sequence homology is low. The macroscopic potentials for the four cytochromes c_3 are summarized in Table I.

Table 1. Macroscopic formal potential of the four hemes in cytochrome c_3, V

	$E_1^{0'}$	$E_2^{0'}$	$E_3^{0'}$	$E_4^{0'}$	$E^{0'}$
DvM	-0.433	-0.469	-0.514	-0.556	-0.501
DvH	-0.462	-0.520	-0.528	-0.580	-0.523
DdN	-0.325	-0.445	-0.489	-0.538	-0.449
Dg	-0.379	-0.470	-0.496	-0.506	-0.463

Electrode potentials vs. Ag/AgCl in saturated KCl
Formal potential, $E^{0'} = (E_1^{0'} + E_2^{0'} + E_3^{0'} + E_4^{0'}) / 4$

Because the heterogeneous electron transfer rate for DvM is very high, it was necessary to employ double pulse galvanstatic analysis to determine this value. Using the technique originally reported by Matsuda (J. Am. Chem. Soc., 81, 5077 (1959)). The overpotential, η, is given by

$$\eta = \frac{RT}{n_a F} \frac{I'}{I^o} \left\{ 1 + \frac{4}{3\pi} \left(\frac{1}{C_o D_o^{1/2}} + \frac{1}{C_R D_R^{1/2}} \right) t_1^{1/2} + \ldots \right\}$$

where I′ is the applied current of the second step. This relationship can be extrapolated to $t_1 = 0$ as shown in Figure 3 to give $I_o = n_a F k^\circ C_o^{1-\alpha} C_R^{\alpha}$

A plot of

$$\log \left(\frac{I_o}{n_a F C_o} \right) \text{ vs } \log k_0 + \log \frac{C_o}{C_R}$$

shown in Figure 4 yields the exchange current, I_o, and hence a standard heterogeneous rate constant (k°) of 1 cm. sec^{-1}.

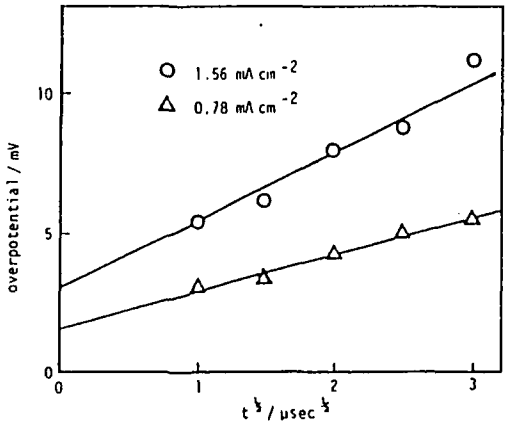

Figure 3

Overpotential vs. square root of the first pulse width of galvanostatic double pulse for 1.11×10^{-4} M cytochrome c_3 from DvM in 0.1 M Tris-HCl, 0.1 M NaCl at pH 7.0.

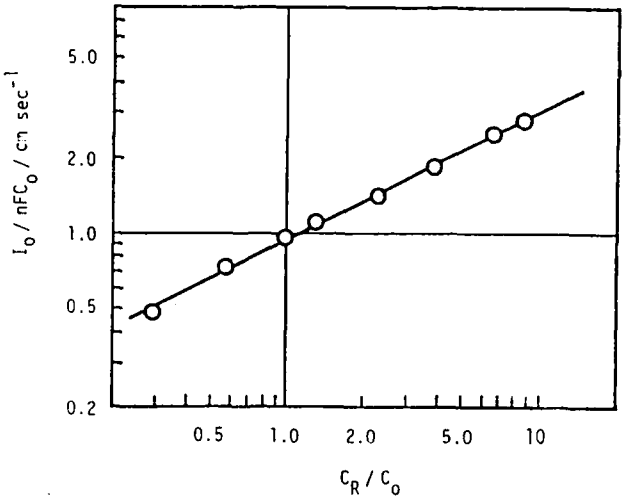

Figure 4 $\log (I_o / nFC_o)$ vs. $\log (C_R / C_o)$ for 1.11×10^{-4} M cytochrome c_3 from *Dv*M in 0.1 M Tris-HCl, 0.1 M NaCl.

WORK PLAN (Year II) The following objectives are envisioned:
1. Complete preparation of adequate quantities of the four cytochromes c_3.

2. Carry out detailed studies of the homogeneous electron transfer reactions of the cytochromes c_3 using stopped flow and flash photolysis.

3. Analyze the kinetic data in terms of the electrostate field maps calculated from the structural data where possible.

4. Complete the studies of carbon monoxide binding.

5. Continue development of a system for expressing site directed mutants of cytochrome c_3.

6. Set up system for simultaneous impedance/spectrophotometric measurements of cytochrome c_3 films. Optimize the geometry of the grid upon which the film is deposited. Measure temperature the dependence of impedance.

7. Carry out the Hall effect experiments. This purpose of these studies is to distinguish between ionic and electronic conductance within the cytochrome c_3 film. It will be particularly important to decide what role, if any, protons play in the conductance process.

8. Begin the spectroscopic studies of Langmuir-Blodgett films.

PUBLICATIONS AND REPORTS (Year I) - None

INVENTIONS (Year I) - None

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